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1. INTRODUCTION:

1.1. Beyond Beauty: Exploring Science of Cosmeceuticals and their Impact on Skin Health and Aesthetics:

Over the years, there has been a shift in the sourcing and formulation of cosmetics from biochemical and chemical ingredients to plant-based sources. This shift can be attributed to several factors, including increasing consumer demand for natural and sustainable products, concerns about the potential health risks associated with certain chemicals, and a growing awareness of their environmental impact. Biochemical and chemical ingredients have been traditionally used in cosmetics due to their effectiveness, stability, and affordability. However, some of these ingredients have now been known to have potential health risks or adverse effects on the environment. For example, certain synthetic fragrances and preservatives have been associated with skin irritations and allergies. In response to consumer preferences and concerns, cosmetic players started to explore plant-based alternatives. Consumers have also favoured vegan or chemical-free cosmetics because- of ethical considerations which include use of animals for testing or use of animal-derived ingredients in cosmetics. Vegan cosmetics are made without the use of animal by-products or derivatives, making them a cruelty-free option. Vegan cosmetics often have a lower environmental impact compared to conventional cosmetics. By opting for vegan cosmetics, individuals reduce their ecological footprint. Individuals are also concerned about the potential health effects of long-term exposure to certain chemicals commonly found in cosmetics. While cosmetic ingredients undergo safety testing, some people prefer to minimize their exposure to synthetic chemicals and choose natural or organic alternatives. **(https://pureecoindia.in/why-vegan-cosmetics-are-trending/)**

There are a number of examples for vegan alternatives like Aloe Vera that is known for its soothing and moisturizing properties, while tea tree oil for its antimicrobial and anti-acne-properties. It is important to note that not all plant-based cosmetics have been claimed as100% natural or organic. However, overall, the shift towards plant-based cosmetics reflects a growing consumer desire for more natural, sustainable, and environmentally friendly options in the beauty industry.

1.2. Transition from cosmetics to cosmeceuticals:

According to the US Food and Drug Administration (FDA), a cosmetic is defined as "a product (excluding pure soap) intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance". Whereas Cosmeceuticals have been defined as cosmetic products with bioactive ingredients purported to have medical benefits. **(Scarlett Puebla-Barragan, 2021)**. Cosmeceuticals are a category of skincare products that bridge the gap between cosmetics and pharmaceuticals. These have been in the market since the last decade and more and are now being preferred over traditional cosmetics. They are designed to improve the appearance and health of the skin by combining cosmetic and pharmaceutical properties. They contain active ingredients that provide therapeutic benefits to the skin, such as anti-aging effects, hydration, sun protection, and brightening. Cosmeceuticals often contain active ingredients such as retinol, hyaluronic acid, peptides, antioxidants (e.g., vitamin C and E), alpha hydroxy acids (AHAs), beta hydroxy acids (BHAs), and botanical extracts. **(Nishu Goyal and Frankline Jerold, 2021).**

1.3. Biotic skin care and microbiome market:

The global microbiome skincare products market size was valued at USD 381.5 million in 2022 and is expected to grow at a compound annual growth rate (CAGR) of 10.9% from 2023 to 2030. North America dominated the industry in 2022 and accounted for the largest share of 34.12% of the overall revenue.

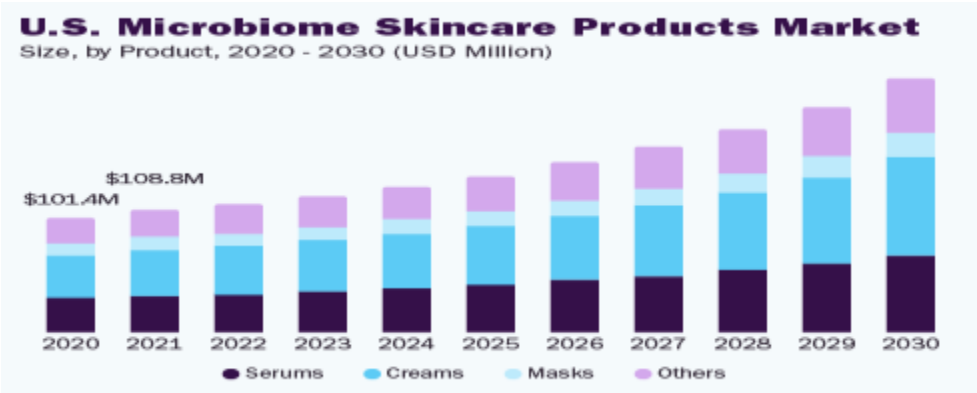

Fig.No.1. U.S.Microbiome Skincare Products Market-

(https://www.grandviewresearch.com/industry-analysis/microbiome-skincare-market-report)

Various skincare products are designed to support a healthy skin microbiome, such as prebiotics, probiotics, microbiome-friendly cleanser, barrier repairing products, and moisturizers. For instance, TULA Life launched its moisturizing, hydrating day & night cream infused with probiotic extracts that provide deep hydration & essential nourishment to the skin. Thus, the growing adoption of these products and the continuously evolving cosmetic industry are anticipated to drive market growth during the forecast period. Research on the skin microbiome is a growing field, and there have been several studies investigating the role of the skin microbiome in skin health and disease. Many research studies have investigated the effects of different types of skincare products on the skin microbiome. For instance, a study published, in 2020in the Cosmetics & Toiletries, which is a Peer-reviewed Cosmetic Science Resource, found that using a microbiome-friendly product, such as Neossance Squalane, led to an increase in the abundance of the diverse microbiome on the skin by 17.9%. Results from such research studies are anticipated to increase attention toward skincare microbiome products, thereby driving market growth. Moreover, several strategic initiatives are undertaken by major players, such as partnerships, collaboration, and licensing agreements, to strengthen their product portfolio and expand their business presence. For instance, BioGaia and Skinome joined forces in September 2022 to research and develop a product that supports the skin microbiome by incorporating live bacteria, naturally improving skin health. This partnership between BioGaia and Skinome shows a growing interest in using probiotics to improve skin health as research continues to reveal the significant impact of the microbiome on overall skin health. Several serums available in the market are formulated to support a healthy skin microbiome, including probiotic & prebiotic serums, Niacinamide serums, and Vitamin C serums. Some of the prominent players in the global microbiome skincare products market include: L'Oreal Paris, BioGaia, Amyris, etc

Some examples of probiotics used as cosmeceuticals are:

PROBIOTICS	**PROPERTIES**	**TYPE OF PRODUCT**
Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus plantarum	Antiacne and antimicrobial properties	Anti-acne creams, Anti-acne face wash.

Lactobacillus paracasei	Imparts antibacterial properties reducing dandruff	Antidandruff shampoos.
Streptococcus thermophilus	Enables skin hydration, rendering an antioxidant effect and pH control	Moisturizing creams and lotions, Anti-aging creams and Anti-wrinkle creams

Table No. 1. Properties of probiotics and incorporation of their lysates in cosmetic products- Probiotics in Skin Care Products- A Review- Nisha K. Kurzekar-2018.

Examples of post biotics in skin care are:

A. The MZ Skin Glow Boost Ampoules also features Lactic Acid, for a gentle exfoliation to brighten dull skin and reduce the appearance of fine lines.

B. MZ Skin Cleanse & Clarify Dual Action AHA Cleanser formulated with a blend of Alpha Hydroxy Acids, namely Lactic Acid, Azelaic Acid, Glucuronic Acid. It also features Papaya Fruit Enzymes to cleanse and exfoliate without irritation for a refined skin texture.

Applying prebiotics to skin helps probiotics thrive so they can: 1. Keep the surface of skin—its first line of defense—balanced, younger-looking, and strong. 2. Offset factors that lead to redness, dryness, and a weakened surface.3. Ward off signs of aging skin.4. Restore a healthy microbiome balance (good bacteria).There are many prebiotics that are beneficial in skin care including:

1.Xylitol, which also functions as a humectant to provide further moisture to the skin.
2.Rhamnose, a prebiotic that has also displayed possible anti-aging benefits.
3.A large group of ingredients known as fructooligosaccharides (including those found in berries).
4.Sugarcane- or chicory root-derived inulin, also offers water-binding property.
5.Alpha glucan oligosaccharide is both a prebiotic and an antioxidant.
6.Fructose and glucose, which deliver hydration-boosting qualities in addition to their prebiotic benefits.

7.Plant extracts from garlic, onions, oats, barley, wheat bran, asparagus, bananas, flaxseed, and their derivatives may also deliver prebiotics to skin. (https://www.paulaschoice.com/expert-advice/skincare-advice/ingredient-spotlight/everything-you-need-know-about-prebiotics-skin.html)

Fig.No.2. PREBIOTICS IN SKIN CARE

Recent research has demonstrated the effectiveness of Xylitol as a substance that inhibits the growth of *Staphylococcus aureus, Staphylococcus epidermidis*, and *Cutibacterium acne* skin microorganisms. A natural sugar alcohol called Xylitol is frequently used in personal care products as a hydrating agent. *S.aureus* and *C. acnes* growth was suppressed by Xylitol; however *S. epidermidis* growth was encouraged. According to these findings, Xylitol can be utilized in personal care products to prevent the growth of bacteria linked with the skin by having unique antibacterial and growth-promoting actions on these bacteria. **(Anglenius, H., &Tiihonen, K. 2020).**

1.4. Ayurveda and Skin care:

Ayurveda is an ancient Indian system of medicine that has been in practice for thousands of years. It is a holistic system that considers mind, body and spirit as interconnected entities and aims to promote overall health and well-being .According to Ayurvedic principles, the skin is the largest organ in the body, and its health is closely tied to the overall health of the body. In Ayurvedic skincare, natural ingredients are used to promote a healthy skin using a wide range of

ingredients, including herbs, oils, and spices, many of which are readily available in Indian homes. In recent years, Ayurveda has gained popularity in cosmetic industry with many companies promoting Ayurvedic skin care products. Some examples of Ayurvedic medicinal plants commonly used in cosmetics: A. Aloe Vera - Moisturizer, Sunscreen & Emollient. B. Calendula officinalis-Wound healing, C. Cichorium intybus-Skin blemishes, D. Curcuma longa-Antiseptic, Antibacterial, Improves complexion, E. Daucus carota -Natural toner and skin rejuvenator. F. Glycyrrhiza glabra - Skin whitening G. Ocimum sanctum - Anti-aging, Antibacterial & Antiseptic H. Rosa damascena - Toning & Cooling I. Rosmarinus officinalis - Skin rejuvenator & Cleansing J. Rubia cordifolia - Wound healing & Anti-aging K. Triticum sativum - Antioxidant, Skin nourisher, and anti-wrinkle. Research had been showed effect of all parts of the Neem tree (leaves, seeds, bark, and stems), Neem oil, and individual Neem-associated phytochemicals have been shown to possess antibacterial, antiviral, antiparasitic, and/or antifungal activities.**(Hazra, J. (2013).**

1.5. Ayurveda Skin Care Market:

The Ayurvedic personal products market will grow at a rate of 16.09% for the forecast period of 2021 to 2028. Ayurvedic personal products market report analyses the growth, which is currently being growing due to increase in the awareness regarding benefits of ayurvedic medicines and personal care products. Ayurvedic products have the ability to well-balance the body and human mind and fight against diseases with minimal or no side-effects. Rise in the adverse effects of consumption of allopathic medicines and chemical-based personal care products is a vital factor responsible for the upliftment of the market growth, also rise in the prevalence of numerous diseases and disorders, skin-related issues, and musculoskeletal disorder and rise in the availability of wide range of natural ingredients-based medicines for almost all health issues and personal care products for skin, hair, and dental products are some of the prime factors among others driving the ayurvedic personal products. North America dominates the ayurvedic personal products market. The major players covered in the ayurvedic personal products market report are Maharishi Ayurveda, Dabur, The Himalaya Drug Company, Sadhu laxmi Ayurveda, Biobaxy, Planet Ayurveda, Arvincare, Natreon Inc and Basic Ayurveda, among other domestic and global players. **(Global Ayurvedic Personal Products Market – Industry Trends and Forecast to 2028-Data Bridge Market Research)**

1.6. The Hidden World of Skin Microorganisms and their role in skin health:

The skin harbours immune cells and is inhabited by billions of resident commensal microorganisms which constitute the so-called skin microbiome shifts. Imbalances in the skin microbiota composition (dysbiosis) are associated with several skin conditions, either pathological such as eczema, acne, allergies or dandruff or non-pathological such as sensitive, irritated or dry skin. The group of organisms colonizing skin can be classified as transitional, temporal and permanent as is with their occurrence based on skin types and across the skin. Transitional they are occasionally appearing. E.g.-Gram positive- *Staphylococcus, Propionibacterium*. Temporal they are present on skin for a short period. E.g. - Gram negative *E.coli* and permanent which appear always on the skin.

Based on the physiological characteristics, human skin sites can be grouped into broad categories: sebaceous or oily (face, chest and back); moist (bend of elbow, back of knee and groin) and dry (volar forearm and palm). Oily skin are known to be dominated by lipophilic, anaerobic coryneform *Propionibacterium* species, whereas bacteria that thrive in humid environments, such as *Staphylococcus* and *Corynebacterium* species, have been preferentially abundant in moist areas, including the bends of the elbows and the feet The production of methane thiol by *Brevibacterium* sp. is supposed to be the principle cause of `foot odor'. Fungi of the genus *Malassezia* are known to predominate in core body and arm sites, whereas foot sites are colonized by a more diverse combination of *Malassezia* spp., *Aspergillus* spp., *Cryptococcus* spp.,*Rhodotorula* spp., *Epicoccum* spp. *Trichoderma* spp**,** *Candida* spp and others. **(Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018)**

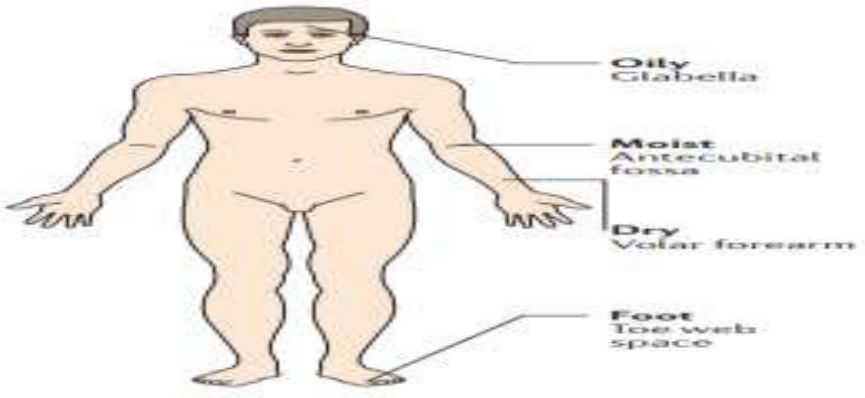

Fig.No.3. Nature Reviews- The human skin microbiome- Allyson L. Byrd, 2018.

Skin commensals have been identified as a potential source of anti-microbial peptides that have a range of benefits for the skin, including anti-aging, moisturizing, and anti-inflammatory properties, as well as the ability to promote collagen production and improve skin elasticity. However, the use of anti-microbial peptides in Cosmeceuticals is a relatively new area of research, and the effectiveness of these peptides in skincare products is not yet fully understood. The goal of this research study is to investigate effect of prebiotics on skin commensals. Skin commensals include *S.epidermidis, S.aureus, S.hominis, S.warneri, S.capitis, S.oralis, Micrococcus luteus, Corynebacterium simulans, C.resistens, C.afermentans, P.acne*, etc however our focus is on *S.epidermidis, S.aureus* and *P.acne*.

S.epidermidis is Gram-positive cocci, nonmotile, non-spore forming facultative anaerobe; it forms white, raised, cohesive colonies about 1–2 mm in diameter after overnight incubation. *S. epidermidis* is found on all skin microenvironments, including dry, moist, sebaceous, and foot regions. *S. epidermidis* is a permanent member of the normal human microbiota. *S. epidermidis* is a commensal of the skin, it may act as an opportunistic pathogen when it breaches the skin surface and enters the bloodstream. It is a leading cause of hospital acquired infections, mostly associated with the use of medical devices in seriously ill or immunocompromised patients. This high incidence is mainly due to its ubiquitous presence on the human skin and its capacity to stick to surfaces of indwelling medical devices during device insertion where it forms biofilms. *S.epidermidis* can rapidly change their phenotypic features. This ability to alternately express the ica ADBC operon is mediated through a mechanism of alternating insertion and excision of the insertion sequence (IS) element IS256 into different sites of the ica ADBC operon, phenomenon, termed phase variation. A family of molecules with multiple functions in *S. epidermidis* are the phenol soluble modulins (PSMs) these are amphipathic, α-helical peptides. Glycerol fermentation of *S. epidermidis* resulted in the production of butyric acid and effectively hindered the growth of a *Staphylococcus aureus*. The *Staphylococcus epidermidis* derived Epidermin was the first lantibiotic that has been shown to be ribosomally synthesized and posttranslational modified. Together with Gallidermin, produced by Staphylococcus gallinarum, they belong to the large class of cationic antimicrobial peptides (CAMPs) that act against a broad spectrum of Gram-positive bacteria. *S.epidermidis* appears to have a particular tendency to produce lantibiotics, peptide antibiotics that contain the characteristic amino acids lanthionine or methyllanthionine. Several lantibiotics of *S. epidermidis* are well characterised, such as

epidermin, Pep5 they share the ability to inhibit methicillin-resistant *S. aureus* (MRSA) **(Christensen, G. J. M., & Bruggemann, H. (2014).**Lipoteichoic acid synthase (ltaS) - LTA is involved in maintaining the structural integrity of the cell wall. It helps stabilize the peptidoglycan layer, which provides rigidity to the bacterial cell wall. LTA promotes initial bacterial attachment to surfaces helps in biofilm formation. It can interact with specific host receptors and extracellular matrix proteins, facilitating bacterial attachment and colonization. S-ribosylhomocysteine lyase (Lux S) -Involved in the synthesis of auto inducer 2 (AI-2) which is secreted by bacteria and is used to communicate both the cell density and the metabolic potential of the environment. The regulation of gene expression in response to changes in cell density is called quorum sensing.

S.epidermidis produces phenol soluble modulins PSMγ and PSMδ that are known to synergize with keratinocyte-derived AMPs and kill opportunistic skin pathogens like MRSA (Methicillin-resistant Staphylococcus aureus) and *Streptococcus pyogenes. S. epidermidis* also makes anti-MRSA quorum sensing inhibitor peptides and a variety of small antimicrobials known as lantibiotics to mediate skin colonization resistance. *S.epidermidi*s lipoteichoic acid and some lipopeptides are known to dampen the inflammatory response to skin injury, accelerating wound healing. Certain *S. epidermidis* strains? can "bloom" and exacerbate Atopic dermatitis or Netherton syndrome skin lesions through production of the EcpA protease. Inflammatory S. epidermidis biofilms that occlude sweat glands have also been shown to exacerbate some AD lesions, NS, Netherton syndrome. **(Brown, M. M., &Horswill, A. R. (2020).**

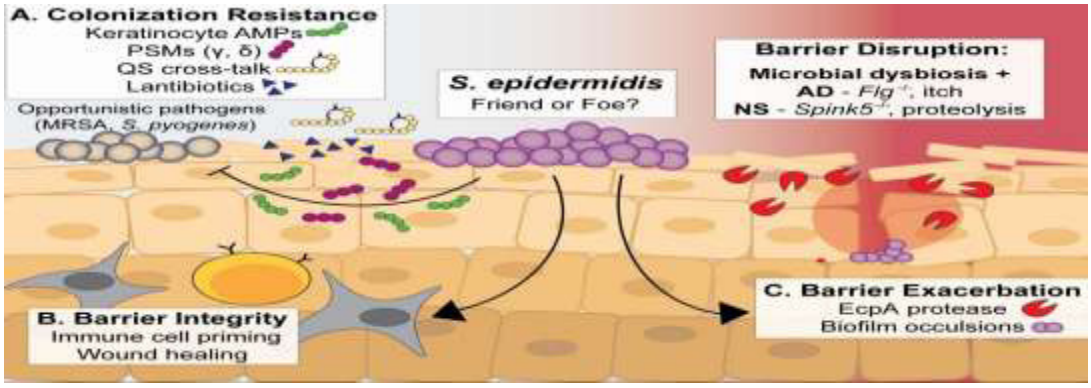

Fig.No.4. PLOS PATHOGENS: *Staphylococcus epidermidis*—Skin friend or foe? - Morgan.M.Brown, 2020.

S.aureus is a gram-positive bacteria tend to be arranged in clusters that are described as "grape-like clusters", non –motile and grows best in an aerobic environment but it can also live in anaerobic condition. It forms circular, 2–3 mm in diameter golden yellow colonies. *S.aureus* is found on human skin, in the nose, armpit, groin, and other areas. *S. aureus* is a transient member of the normal human microbiota. Human skin produces several AMPs that are effective against *S. aureus*. One of the most well-known skin-derived AMPs is called Cathelicidin. Cathelicidin are α-helical, amphipathic and cationic. Cathelicidin is constitutively expressed in neutrophils and has potent bactericidal activity against *S. aureus*. It is also called LL-37, referring its first two amino acids and total length of 37 amino acids. **(Sunhyo Ryu, Peter I Song, 2014)**.It works by disrupting the bacterial cell membrane, leading to the death of the bacteria. In addition to cathelicidin, there are other skin-derived AMPs that can inhibit the growth of *S. aureus*. These include defensins, which are small cationic peptides that also disrupt bacterial cell membranes causing leakage of cellular contents and ultimately leading to the death of the bacterium. *S. aureus*, however, has developed several mechanisms to evade or resist the antimicrobial actions of defensins and other host defense peptides. **(Sunhyo Ryu, Peter I Song, 2014)**.These mechanisms include the production of enzymes that can degrade or inactivate defensins, alterations in the bacterial cell wall to reduce defensin binding, and the formation of biofilms, which provide protection against the immune system and antimicrobial agents. Lipoteichoic acid is an essential component of the *S.aureus* cell envelope and an attractive target for the development of vaccines and antimicrobials directed against antibiotic-resistant Gram-positive bacteria such as methicillin-resistant *S.* aureus. Accessory gene regulator (agr) - Essential for the production of a quorum sensing system signal molecule, the auto inducing peptide (AIP). This quorum sensing system is responsible for the regulation of the expression of virulence factor genes. Staphylococcal protein A (spA) is an important virulence factor which enables *S. aureus* to evade host immune responses.

***P.*acne** is gram positive, rod shaped bacteria, anaerobic microorganism, non-motile, predominantly in the sebaceous gland-rich areas of the skin. *P.acne* is a transient member of the normal human microbiota. It forms circular and opaque, white1-2 mm in diameter colonies. *P. acnes*, get its name from its ability to produce propionic acid, a short-chain fatty acid (SCFA), during fermentation. A number of SCFAs are commonly found in the skin and in the secretion of skin glands, such as sweat. *P. acnes* are a probiotic bacterium and can convert glycerol into

propionic acid by fermentation. Propionic acid effectively suppressed the growth of *Staphylococcus aureus*. Glycerol, a carbon source for bacterial fermentation, is a naturally occurring metabolite and humectant found in the human skin. It has been reported that glycerol can be produced endogenously by breakdown of triglycerides by sebaceous gland-associated lipase in the human skin. Fatty acids generated by lipase activities retard the development of transient microorganisms on skin surfaces, e.g. *S. pyogenes and S. aureus*. The accumulation of propionate and other short-chain fatty acids (SCFAs) can lower the pH and thus inhibit the growth of other bacteria, which might be beneficial for the host since it would prevent the colonisation of (more) harmful pathogens. Propionic acid and its salts are commonly used in the preservation of foods and manufacture of cellulose, perfumes, herbicides, and pharmaceuticals. **(Y. Wang1, A. Dai1, S. Huang2, 2014).** Lipoyl synthase (Lip A) -An enzyme responsible for the synthesis of lipoyl-ACP. Lipoyl-ACP is a cofactor involved in several metabolic pathways, including the oxidative decarboxylation of pyruvate, which is an important step in energy production. Fibronectin Binding Protein A (fbp A) - Fibronectin is a glycoprotein present in the extracellular matrix, which provides structural support to cells and plays a role in wound healing and tissue repair.

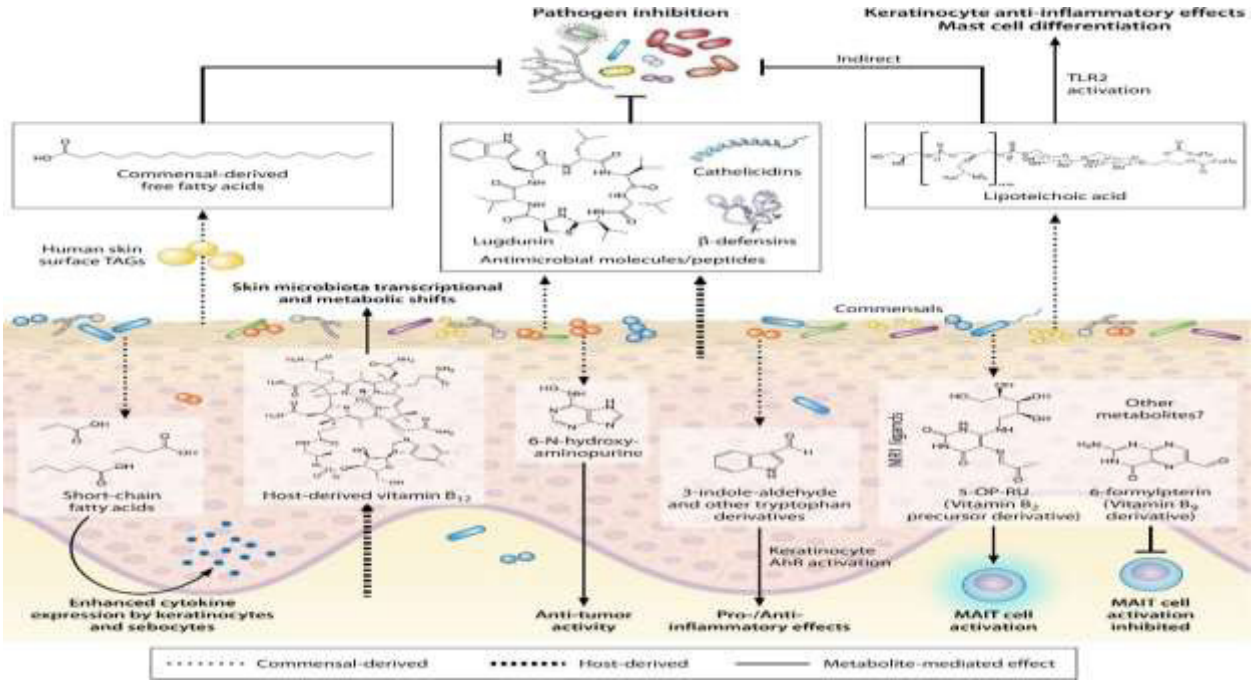

Fig.No.5. Living in Your Skin: Microbes, Molecules, and Mechanisms- Mary Hannah Swaney, 2021.

The aim of the present work is to use standard prebiotics comparing them to widely used prebiotics like Xylitol and observe their effect on skin commensals like *S.epidermidis, S.aureus* and *P.acne.* Our goal is to boost *S.epidermidis* growth and inhibit growth of S.aureus and *P.acne.*

2. AIMS OF STUDY:

1. Prebiotics selection- Selection of prebiotics like Triphala,/ Ambe Haldi,/ Gulwel,/ Ashwagandha etc documented in ayurveda for prebiotic activities.

2. Comparison of onion,/ garlic,/ flaxseed,/cauliflower activity of selected prebiotics with commercially used prebiotics like Xylitol, Rhamnose, etc.

3. Exploring the effect of the above selected prebiotics on growth and biochemical profile of skin commensals like *S.epidermidis* and or *S.*aureus and or *P.acne*.

4. Studying regulation of genes like lta S and or lux S and or agr and or spA and or lipA and or fbp A in the selected commensal/s in response to prebiotics.

3. OBJECTIVES OF STUDY:

1. Prebiotics like Triphala, Ambe haldi, Gulwel and Ashwagandha that have been reported to have skin enhancing properties would be used for the present study. Also prebiotics like onion, garlic, flaxseed and cauliflower would also be used. Extraction of actives from selected prebiotics would be performed using suitable solvents for example methanol, Petroleum ether.

2. Agar well diffusion method would be used to observe the effect of .prebiotics on growth/ inhibition of the selected organisms viz *S.aureus* and or *S.epidermidis* and or *P.acne*. MIC and MBC would be performed.

3. Profiling of prebiotics and microbial actives using gas chromatography or GC- HRMS mass spectrometry. Study of production of Anti-Microbial Peptides/ actives by *S.epidermidis*- like Epidermin and or Gallidermin and or *S.aureus*- Lipoteichoic acid and or *P.acne*- Lipase and or Protease.

4. Gene expression studies to observe changes in crucial gene regulation using RT-PCR. Eg- *S.epidermidis*- lta S and or lux S, and or *S.aureus*- agr and or spA and or *P.acne*- lipA and or fbp A.

4. STUDY BACKGROUND AND SIGNIFICANCE OF STUDY:

Biotic skin care is the broad term which has been categorized by the products infused with biome-specific ingredients, like prebiotics-feed bacteria, probiotics-add bacteria, and postbiotics-use bacterial compounds.Prebiotics are food for the good bacteria, they are essentially foods consumed by probiotics**.** Probiotics defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Postbiotics is a 'preparation of inanimate microorganisms and/or their components that confers a health benefit on the host.

Scientists in Pune and Bengaluru studied the unique composition of skin and oral microorganisms of Indians, as well as their distinct immune responses, which could give clues into why the Covid death rate in India was relatively lower than among the European and American populations.Their study indicated that the relatively low Covid mortality in India perhaps had something to do with protection provided by the unique microbiota (community of microorganisms) on the skin, as well as in the oral and nasal cavities of Indians. Also, Indian food habit & food ingredients was said to have a role in lowering the severity & high death rate from COVID-19 in Indians. (https://timesofindia.indiatimes.com/covid-nov-3-2021/coronabytes/msid-87498412.cms).

Some medicinal plants have been used in cosmetic skin care like Calendula, also known as marigold, has been used for centuries for its healing properties. It contains prebiotic polysaccharides that can nourish the skin's microbiome and promote a healthy balance of bacteria. Calendula is often used in skincare formulations for its calming and soothing effects on the skin. Chamomile is a gentle herb that has anti-inflammatory and antioxidant properties. It contains prebiotic compounds that can help balance the skin's microbiome, soothe irritation, and promote skin healing. Chamomile is often used in skincare products for sensitive or reactive skin. . Ashwagandha contains compounds like flavonoids and withanolides, which are believed to possess antioxidant properties. Ashwagandha is used to calm inflamed and stressed skin. When applied topically it also helps to support collagen production, which can help prevent visible signs of premature ageing. Ambe Haldi contains compounds like curcuminoids, which are antioxidants that help neutralize free radicals in the skin. This antioxidant activity may help protect the skin from oxidative stress, which can contribute to premature aging, wrinkles, and dull complexion. Ambe Haldi has been traditionally used for wound healing purposes. Its anti-

inflammatory and antimicrobial properties may help support the healing process and prevent infections in minor cuts or abrasions. Curcuminoids in Ambe Haldi possess anti-inflammatory properties that may help reduce redness, irritation, and swelling of the skin. It may be beneficial for people with inflammatory skin conditions like acne, eczema, or psoriasis. Triphala is rich in antioxidants, such as vitamin C and flavonoids, which help neutralize free radicals and protect the skin from oxidative stress. This can help in preventing premature aging and maintaining healthy skin. The vitamin C content in Triphala supports collagen synthesis, a protein essential for maintaining skin elasticity and reducing the appearance of fine lines and wrinkles. Triphala can be used as a natural cleanser and toner for the skin. It helps remove impurities, excess oil, and dead skin cells, leaving the skin refreshed and rejuvenated. So our approach is to use such medicinal plants powder as prebiotics to improve skin health.

5. INTERNATIONAL AND NATIONAL STATUS:

1. **Allyson L. Byrd1–4, Yasmine Belkaid3,5 and Julia A. Segre1- March 2018**-Had recent insights into skin microbial communities, including their composition in health and disease, the dynamics between species and interactions with the immune system, with a focus on *Propionibacterium acnes, Staphylococcus epidermidis and Staphylococcus aureus.*

2. **Elizabeth A. Grice and Julia A. Segre- April 2011-** An enhanced understanding of the skin microbiome is necessary to gain insight into microbial involvement in human skin disorders and to enable novel antimicrobial therapeutic approaches for their treatment.

3. **John Wiley & Sons Ltd- November 2019**-overview on the current knowledge on the skin microbiome, the currently available sampling and analysis techniques as well as a description of current approaches undertaken in the skincare segment to help restoring and balancing the structure and functionality of the skin microbiota.

4. **Nisha K. Kurzekar, Dr. D. D. Wasule-2018**- Rather than including live bacteria cultures, many of the probiotic skin care formulae use bacteria fragments or metabolites. In near future, some brands using live bacteria might emerge. Recently there have also been an increasing number of skin care products entering the market.

5. **Morgan M. Brown, Alexander R. Horswill- February 2023**- While the field has historically assumed that all *S. epidermidis* isolates behave similarly, emerging evidence suggests that colonization by specific strains of *S. epidermidis* can either help or hurt the skin barrier depending on the context. We challenge the current dogma that "all *S. epidermidis* strains behave equally".

6. **G.J.M. Christensen and H. Bruggemann- 13 June 2013-***S. epidermidis* is a causative agent of hospital-acquired infections, mostly associated with the use of medical devices. *P. acnes* is suspected to be of major importance in the pathogenesis of acne and also in a number of other opportunistic infections. In this review we will present bacterial factors and traits of these two key members of our skin microbiota and discuss how they contribute to mutualistic and parasitic properties.

7. **Cindy Le Bourgot, Claire Meunier- 2022-**Effects of short chain fructo-oligosaccharides on selected skin bacteria- The objective of the current study was to evaluate the effects of short chain fructo-oligosaccharides (scFOS) from sugar beet (DP 3–5), well-recognised prebiotics, on some representative bacterial strains of the skin microbiota.

8. **Heli Anglenius and Kirsti Tiihonen- 14 February 2020**-Evaluation of Xylitol as an agent that controls the growth of skin microbes: *Staphylococcus aureus, Staphylococcus epidermidis,* and *Cutibacterium acnes*-Xylitol has specific antimicrobial and growth-promoting effects on skin associated bacteria, and can be used in personal care products to control the growth of these bacteria.

9. **G.J.M. Christensen and H. Bruggemann- 13 June 2013**-Bacterial skin commensals and their role as host guardians- The elucidation of roles of *S.epidermidis* and *P.acne* in health-promoting or disease-causing processes could lead to new prophylactic and therapeutic strategies against skin disorders and other *S. epidermidis/P. acne*s-associated diseases, and increase our understanding of the delicate interplay of the skin microbiota with the human host.

10.**Arun Balasubramaniam, PrakosoAdi- Scientific Reports-2020**- Repurposing INCI-registered compounds as skin prebiotics for probiotic *Staphylococcus epidermidis* against UV-B-Exploring new indication of LCC for promoting S. epidermidis against UV-B provided an example of repurposing INCI registered compounds as skin prebiotics.

6. STUDY HYPOTHESES:

The overreaching goal of this study is to boost the growth of *S.epidermidis* and inhibit *S.aureus* and *P.acne.* This is proposed to be mediated through the use of prebiotics. Effect of prebiotics on skin commensals like *S.aureus, S.epidermidis,* and *P.acne* has been observed in our preliminary studies. Prebiotics like Gulwel and Ashwagandha boosted growth of *S.epidermidis* whereas they were inhibitory to *S.aureus*. Triphala and Ambe Haldi inhibited *P.acne*. Also prebiotics like onion boosted growth of *S.epidermidis* and garlic and flaxseed were inhibited by *S.aureus*.

It is however essential to understand the mode of action of these organisms that enable them to compete with each other. It is equally essential to understand if this could be boosted by the use of the selected prebiotics.

7. STUDY RATIONALE:

While it is well known that the skin is home to a diverse population of commensal microorganisms, their precise role in maintaining skin health and preventing disease is yet to be fully understood. Phenotypic variation, environmental changes, dietary habits are known to affect the composition of the skin microbiota. Although per/pro and post biotics have been shown to modulate the composition of the gut microbiota, their mechanism of action on skin commensals is so far quite unknown. It is unknown how biotics is metabolised by skin commensals, how they impact the structure and function of the microbial community, and how these changes translate into observable effects on the skin. Currently, there is no standardised protocol for studying the effect of biotics on skin commensals, making it difficult to compare results across studies. Variables such as biotic concentration, time of exposure, and the skin commensals employed in research can all have an impact on the results, and more standardised techniques are required to increase the reliability and repeatability of results. Although some biotics have been shown in animal models to have skin-beneficial effects, there is limited clinical evidence to support their use in humans. More research is needed to find the best biotic compositions, doses, and application methods for boosting human skin health. Finally, much remains to be discovered about the complex interactions that exist between the host and skin commensals, as well as how prebiotics can be employed to control these interactions for therapeutic purposes. More study is needed to understand the underlying processes and routes of host-microbe communication, as well as to uncover novel targets for biotic intervention.

Understanding the interactions between skin commensals in presence and absence of plant actives can provide insights into potential cosmeceuticals targets for enhancing skin health and preventing infections. A review by Allyson L. Byrd1–4, Yasmine Belkaid- 2018-Nature-The Skin Microbiome discussed about the role of *S.epidermidis in* degrading a protein crucial for *S. aureus* biofilm formation. *S.hominis* inhibiting S.aureus growth by production of lantibiotic. Whereas *S.lugdunensis* inhibiting *S. aureus* biofilm growth by production of antibiotic lugdunin and *P.acne inducing S.aureus* biofilm by production of small molecule coproporphyrin III.

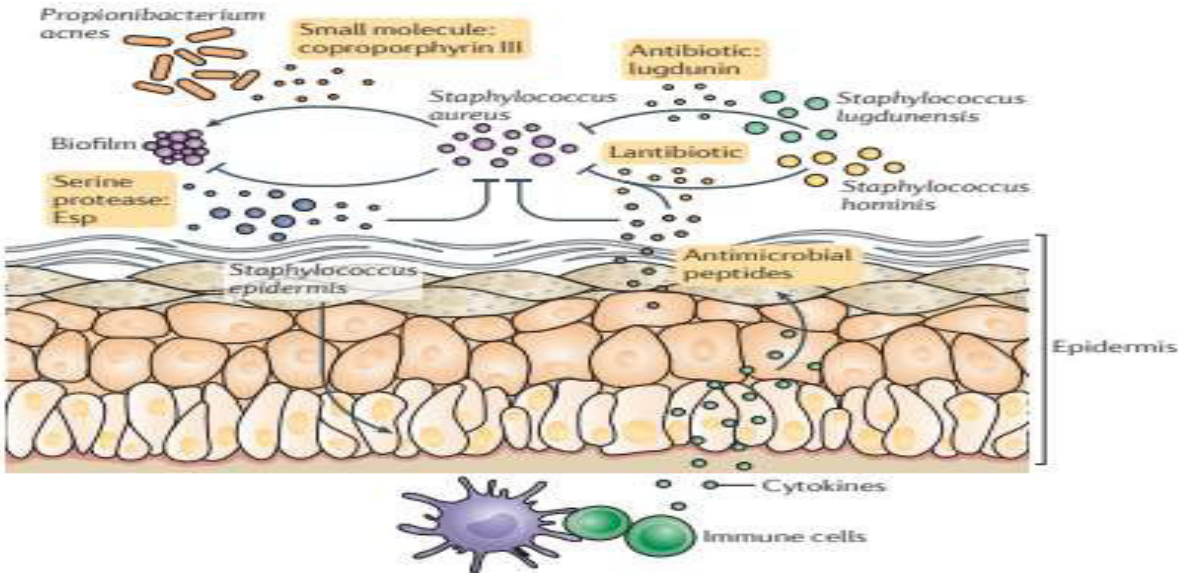

Fig.No.6. Nature Reviews Microbiology: The human skin microbiome- Allyson L. Byrd, 2018.

8. RESEARCH METHODOLOGY:

1. Selection and identification of the skin commensals like *S.epidermidis, S.aureus* and *P.acne.* and prebiotics like Triphala, Ambe Haldi, Gulwel and Ashwagandha and or onion, garlic, flaxseed and cauliflower based on literature. Prebiotics were purchased from ayurvedic shops and local market. Skin commensals were purchased from MTCC.

2. Extraction of prebiotics as per standard methods- A. 1 g sample medicinal plant formulation/powder was dissolved in 10 ml methanol and kept on magnetic stirrer for 2 days. B. Sample was sonicated for 20 to 40 minutes. C. Centrifuged at 7000 rpm for 20 minutes. D. Supernatant was collected in pre weighed petriplate and kept overnight. E. Sample was dissolved in Dimethyl Sulfoxide (DMSO) for further experimentation and storage. (Berhan Begashaw1, Bharat Mishra- Methanol leaves extract Hibiscus micranthus Linn exhibited antibacterial and wound healing activities- BMC Complementary and Alternative Medicine (2017)).

3. Using extract Agar Well Diffusion would be carried to observe zone of inhibition and further Minimum Inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC) will be performed.

4. Production of Anti-Microbial Peptide by *S.epidermidis*- Epidermin, Gallidermin, Lantibiotic, *S.aureus*- Lipoteichoic acid and *P.acne*- Lipase, Protease by using vacuum filtration system and making extraction solvent (Acetonitrile: methanol: water: 2:2:1).

5. Metabolite profiling of microbial metabolites that facilitate anti-ageing and anti-wrinkle properties using gas chromatography or mass spectrometry.

4. Gene expression studies to observe changes in crucial gene regulation using RT-PCR. Eg- *S.epidermidis*- lta S, lux S, *S.aureus*- agr, spA and *P.acne*- lipA, fbp A.

9. STATISTICAL PLAN:

The biochemical tests anti-microbial, anti-ageing, anti-wrinkle testing and related experiments will be performed in triplicates if not quadruplet (n=4) to obtain statistically significant data. Results will be analysed using proper statistical techniques like ANOVA.

10. LIMITATIONS OF STUDY:

The presence of millions of bacteria, viruses, and fungi on the skin is a natural part of our microbial ecosystem. However, when it comes to addressing skin-related concerns, such as maintaining a healthy skin microbiome or treating specific conditions, the focus is predominantly on bacteria rather than viruses and fungi. This distinction arises because bacteria have been extensively studied and are known to play a crucial role in skin health and disease. The proposed work aims to explore the use of prebiotics, which are substances that promote the growth and activity of beneficial bacteria, including live bacterial cultures, on the skin. While prebiotics have shown potential in improving skin health, there may be challenges in gaining acceptance. One approach is to educate the public about the importance of maintaining a balanced and diverse skin microbiome for overall skin health. Emphasizing the beneficial functions of certain bacteria, such as their ability to inhibit the growth of harmful pathogens or promote skin barrier integrity, can help overcome the negative perception of bacteria.

11. EXPECTED OUTCOME OF STUDY:

Use of cosmetics has often been influenced by the prevailing societal norms, cultural practices, and technological advancements of their time. For example, ancient Egyptians used natural ingredients like kohl and henna for beauty enhancement, while Renaissance-era cosmetics emphasized pale skin and red lips. Looking back at these historical influences can provide inspiration for future cosmetic innovations. Consumers worldwide are going green, and this is especially true in the cosmetics market. Over the last few years, growth in the beauty market has been driven by products that use natural or herbal components, and the market share of natural-based products has been increasing gradually. Companies are feeling pressure to focus on sustainability and reduce their environmental footprints while consumers are more aware and better informed than ever before. They are becoming conscious of the ingredients that go into the products they use and are averse to chemicals that are known to cause side effects. On the supply side, companies are engaging in advanced research of plant-derived peptides, encapsulated actives, active plant stem cells, complex extraction processes and clinical testing to deliver products that are acceptable to the well-informed clients. The India market has been traditionally inclined toward natural products for their beauty needs, and Indians have a history and knowledge of using natural products. While consumers in the rest of the world have to be educated about the benefits of natural herbs, this knowledge is well-inherited in India from generations. The need is to translate the ancient ayurvedic recipes into modern, easy-to-use formulation correlating their biochemical and molecular effects. (Shilpa Sevatkar K Sharma Reetu, G. B. (n.d.).Cosmetology in Ayurveda –A Review)

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